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TEMPERATURE AS A FACTOR IN THE INFECTION OF COTTON SEEDLINGS BY TEN PATHOGENS

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THE PLANT DISEASE REPORTER

PLANT DISEASE EPIDEMICS AND IDENTIFICATION SECTION

Crops Research Division — Plant Industry Station, Beltsville, Maryland

TEMPERATURE AS A FACTOR IN THE INFECTION OF COTTON SEEDLINGS BY TEN PATHOGENS[1]

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Abstract

Cotton seeds naturally infested by or artifically inoculated with ten fungi were grown in culture at 18°, 21°, 24°, 30°, 33°, and 36° C. The temperature range through which the several fungi infected the seedlings was as follows: Ascochyta gossypii, 18°-27°; Botryodiplodia phaseoli, 18°-36°; Colletotrichum gossypii, 18°-33°; Fusarium moniliforme, 18°-36°; Fusarium oxysporum f. vasinfectum, 18°-36°; Pythium ultimum, 18°-27°; Rhizoctonia solani, 18°-33°; Thielaviopsis basicola, 18°-30°; Verticillium albo-atrum, 18°-21°; and Xanthomonas malvacearum, 21°-36°. The latter produced lesions only on the cotyledons. Botryodiplodia phaseoli was a more aggressive parasite of the cotyledons than of the hypocotyls.

The characteristics of the lesions produced by the several fungi are described.

INTRODUCTION

A number of pathogens have been shown to infect cotton seedlings and cause damping-off (4, 37, 50, 62). The incidence of the diseases caused by each of these pathogens varies greatly from year to year, and they are not always controlled by the fungicides used for seed treatment. This is especially true when soil conditions are favorable for seedling infection by soil-inhabiting pathogens. The discovery of more effective fungicides for their control is dependent upon detailed information of the environmental conditions under which these pathogens infect cotton seedlings. Temperature was chosen for the environmental variable to be studied, since it is readily controlled and is known to be an important factor in determining the amount of seedling infection when soil moisture is adequate.

This paper presents data on the infection of cotton seedlings by 10 pathogens through a temperature range of 18°-36°C. Descriptions of the lesions caused by several pathogens are also included as an aid to identification of the causal pathogens under field conditions.

METHODS

The seedlings were grown in sand culture at controlled temperatures by essentially the same method as that described previously (8). Sufficient nutrient solution was added to the sand cultures to bring the amount of water to 80 percent of the field capacity. Additional water was added to maintain this moisture content when approximately 15 percent of the water had been lost by evaporation. The time required for this 15 percent reduction varied from 4 to 8 days, the shorter time being required at the higher temperatures. Since evaporation tended to lower the temperature of cultures below that of the air, the latter was maintained at a temper-

[1] Technical Contribution No. 271 of the South Carolina Agricultural Experiment Station, Clemson, South Carolina.

ature which would provide the desired culture temperature at seed depth, or 2.5 cm. The difference in the air and culture temperatures at 18°C was less than 0.25°; and this difference increased regularly at the higher temperatures to a maximum of 1.5° at 36°. After emergence the seedlings were irradiated for 16 hours each day with fluorescent lamps.

The pathogens used and their origin are indicated in Table 1. From one to three isolates of the several pathogens were used. When more than one isolate was used, the cultures of each were mixed in a mechanical blender, as indicated later. No attempt was made in these studies to assure that the isolates used were derived originally from a single spore or hypha. The use of mixed cultures of the same pathogen in these studies was considered advisable, since the main objective was to ascertain the temperature range over which strains of a given pathogen might infect seedlings and produce typical lesions for that pathogen.

Each isolate used in this study, except Verticillium albo-atrum, had been reisolated from an infected cotton seedling, although it may have been obtained originally from a host other than cotton. All isolates were obtained from sections of infected hypocotyls that were first immersed for 10 to 20 seconds in 95% ethanol and then for 1 to 3 minutes in a hypochlorite solution that contained 2%-3% free chlorine; after which they were cultured on non-nutrient agar. This same technique was used throughout the study to ascertain whether or not the fungus used for inoculation had infected the seedlings.

Five-day-old cultures of the pathogens grown on cornmeal-raisin agar slants at room temperature were used to inoculate the seeds. The inocula were prepared by scraping the mycelium from the surface of the agar and macerating in a blender for 20 seconds. The seeds were usually inoculated with this mycelial suspension by placing one drop on each seed after it had been placed in the sand culture. In some instances, as indicated, the seeds were soaked in the suspension immediately before the seed was placed in the sand culture. These methods of inoculation added only minute amounts of organic matter to the cultures.

Special care was taken to assure that the seeds used in these studies were not internally infected by fungi. Consequently, acid-delinted seed from which the floaters (7), about 30 percent, had been removed were used in the tests, except when otherwise indicated. Fuzzy seed naturally infested by the anthracnose fungus were used in one series of tests. In the first set of tests with each pathogen, a lot of Stoneville-2B seed of 95 percent viability was used. All results with this variety were verified by later tests in which seed of the variety Coker-100W was used. Since comparable results were usually obtained with both varieties, only occasional reference will be made to them. As will be noted later, seed of several other varieties were used in tests in which it was necessary to use a variety of known susceptibility to infection.

Generally, 5 sand cultures of 32 seeds each, as used in other studies (8), were used in a test of each pathogen at a given temperature. For convenience in making comparisons, all data have been adjusted to a 100-seed basis. Consequently, the numbers given are percentages. Over the temperature range in which infection was obtained, the tests were repeated two or three times. After 14 days, all cultures with more than 10 viable seedlings were thinned to that number by a systematic removal of plants so as to leave the remaining seedlings uniformly distributed throughout the culture. Sections of the hypocotyls of the removed seedlings were plated on a non-nutrient agar. All seedlings alive after 21 days were plated in the same manner to ascertain the absence or presence of infecting fungi. The numbers of healthy and infected seedlings after 21 days and the numbers of such seedlings removed previously were used to estimate the total numbers of healthy and infected seedlings after 21 days. In making these estimates it was assumed that the healthy seedlings removed after 14 days would have shown the same percentage of infection as the 10 or fewer healthy seedlings left in the individual culture from the 14th to the 21st day.

RESULTS

Rate of Seedling Emergence

The relative rate of emergence of uninoculated seeds at the several temperatures, as indicated by the coefficient of emergence (35)(Table 2) was not greatly different from that reported previously (6, Fig. 1.) At 18°C in the uninoculated cultures, the emerged cotyledons were still yellow or pale green after 14 days; and in the various cultures from 25 to 50 percent of them were still chlorotic after 21 days. At 21°, some cotyledons were chlorotic after 11 days, but there was no evident chlorosis after 14 days.

As in the earlier study of the temperature relations of cotton seedlings (6), the appearance

Table 1. Origin of pathogens used in studies of temperature relations.

Pathogen	Culture No.	Host	Locality	Supplier
Ascochyta gossypii Syd.				
	1	Cotton	S. C.	C. H. Arndt
	2	Cotton	N. C.	S. C. Lehman
	3	Okra	N. C.	S. C. Lehman
	4	Soybean	N. C.	S. C. Lehman
Botryodiplodia phaseoli (Maubl.) Thir.				
	5	Cowpea	Ga.	E. S. Luttrell
	6	Cowpea	S. C.	J. K. Armstrong
	7	Cotton	S. C.	C. H. Arndt
Colletotrichum gossypii Southworth[a]				
	8	Cotton	S. C.	C. H. Arndt
	9	Cotton	S. C.	C. H. Arndt
	10	Cotton	S. C.	C. H. Arndt
	11	Cotton	Miss.	C. H. Arndt
Fusarium moniliforme Sheldon				
	12	Crowder pea	S. C.	J. K. Armstrong
	13	Cotton boll	Okla.	W. W. Ray
	14	String bean	Idaho	W. W. Ray
	15	Corn	S. C.	C. H. Arndt
	16	Corn	Miss.	C. H. Arndt
	17	Cotton	S. C.	C. H. Arndt
Fusarium oxysporum f. *vasinfectum* (Atk.) Snyd. & Hans.				
	18	Cotton	S. C.	J. K. Armstrong
Pythium ultimum Trow				
	19	Pepper	S. C.	C. H. Arndt
	20	Cotton[b]	S. C.	C. H. Arndt
	21	Cotton[b]	S. C.	C. H. Arndt
	22	Guayule	Calif.	W. A. Campbell
Rhizoctonia solani Kuehn[c]				
	23	Cotton	Texas	C. H. Arndt
	24-	Cotton	S. C.	C. H. Arndt
	25	Cotton	S. C.	C. H. Arndt
Thielaviopsis basicola (Berk. & Br.) Ferr.				
	26	Tobacco	Canada	G. B. Lucas
	27	Tobacco	N. C.	G. B. Lucas
	28	Cotton	Miss.	J. T. Presley
Verticillium albo-atrum Reinke & Berth.				
	29	Cotton	Texas	P. J. Lyerly
	30	Cotton	N. C.	S. C. Lehman
	31-	Cotton	Miss.	J. T. Presley
Xanthomonas malvacearum (E. F. Sm.) Dowson				
	32	Cotton	Texas	L. S. Bird
	33	Cotton	Okla.	L. A. Brinkerhoff

[a] Cultures 8, 9, 10, and 11 were derived from lesions that developed on cotton seedlings grown in steamed sand cultures from seed that had been treated with Ceresan-M; hence, presumably derived from seeds internally infected by this fungus.

[b] Isolated from seedlings grown in river sand obtained near Clemson, S. C. (20); and from soil obtained near Charleston, S. C. (21)

[c] Isolated from seedlings grown from acid-delinted seed in soil received from Greenville, Texas (23), in river sand obtained near Clemson, S. C. (24), and in soil near Charleston, S. C. (25).

Table 2. Effect of indicated temperatures on the emergence of cotton seedlings in uninoculated cultures.

Temperature °C	Emergence: Total percent	Days required for Initial	Days required for Total	Coefficient of emergence[a]
18	64	7	14	11
21	88	5	8	18
24	89	4	5	23
27	85	3	4	32
30	90	2	3	40
33	90	2	3	40
36	86	2	3	42

[a] As calculated by Kotowski (35), or the coefficient of velocity of

$$\text{emergence} = \frac{\text{Total emergence at end of period}}{\text{Sum of (each daily emergence increase x days since planting)}} \times 100$$

of the seedlings indicated that a sustained temperature of 33°C was too high for best seedling development. At 27° and 30° after 17 days, the first foliage leaves had reached a length of 1 to 2 cm; while at 33°, they were still mere rudiments after 21 days. From 1 to 2 days longer were required for the cotyledons to expand fully at 33° than at 30°, although at both temperatures the seedlings emerged at about the same time. At 36°, the expansion of the cotyledons was still further delayed. Only 60 percent had emerged from the testas after 8 days, and 20 percent had not emerged after 14 days, but all were fully expanded after 21 days.

Ascochyta gossypii[2]

Acid delinted seed were inoculated by immersion for 5 minutes in a mycelial suspension of isolate #1 (Table 1) of Ascochyta gossypii. At 18°C there was no emergence, at 30° emergence was 97 percent and no seedlings were infected, Table 3. At 21°, 24°, and 27°, the first seedlings were killed after 9, 7, and 11 days, respectively; after 14 days the percentages of normal seedlings were 23, 16, and 35 respectively; and after 21 days, 2, 6, and 29, respect-

Table 3. Percent seedling emergence, number of seedlings alive after 14 and 21 days, and the day of culture on which the first of the emerged seedlings was killed for seeds inoculated with Ascochyta gossypii and cultured at the temperatures indicated.

Temperature °C	Percent seedling emergence	Number of seedlings alive after 14 days	Number of seedlings alive after 21 days	Number of days to death of first seedling
18	0	0	0	0
21	52	23	2	9
24	79	16	6	7
27	88	35	29	11
30	97	97	91	0
33	90	90	90	0
36	90	90	90	0

[2] The writer wishes to express his indebtedness to W. B. Boykin and Charles Brown for assistance in obtaining the data herein reported.

ively. Thus, the fungus may cause severe seedling loss at 21°, 24°, and 27°C. At 18° and 24°, other cultures were inoculated with an aqueous suspension of spores which were obtained from Petri-dish cultures on which pycnospores had been produced. When the latter were grown concurrently with cultures inoculated with the mycelial suspension, comparable results were obtained, except that 2 to 3 days longer were required for the death of the first seedling and there was a slightly greater number of surviving seedlings.

In a supplementary test, isolates of A. gossypii obtained from cotton, okra, and soybean in North Carolina were compared with the South Carolina isolate at 21°C. The North Carolina cotton isolates appeared slightly more virulent than the one from South Carolina. The okra and soybean isolates did not infect the cotton seedlings. More recently published results (17, 30, 61) would indicate that the lack of virulence of the latter two isolates may not be characteristic of all isolates from these hosts.

The first symptom of infection was the appearance of a reddish-brown lesion on the hypocotyl at or just below the surface of the soil. In 2 to 3 days this lesion enlarged to involve the entire circumference of the hypocotyl but with little or no extension of the lesion above the soil. Four to 5 days after the appearance of the lesion, the seedling would fall over but otherwise would appear healthy and unwilted. At this stage of infection, the cortex of the hypocotyl and root would be thoroughly rotted and would slough off readily. The seedlings did not wilt until 2 to 3 days later. Presumably, the cortex is seriously affected earlier than the stele.

Although Ascochyta gossypii is undoubtedly the cause of occasional severe injury to young cotton plants (2, 19, 29, 43, 56), the report by Smith (56) is the only one which indicates that it is a possible cause of seedling losses. It was not reported by Weindling et al. (62). It has not been isolated from diseased seedlings by the writer. The fungus may have infected a somewhat larger number of diseased seedlings than the reports indicate, since it grows more slowly on most culture media than most of the other fungi associated with hypocotyl lesions. Although this fungus may not be the primary cause of seedling losses, cotyledonary infection by it may predispose the seedlings to severe injury by other pathogens. In one season in which Ascochyta and thrips had injured the cotyledons, the writer observed an unexpected and widespread destruction of cotton seedlings by Rhizoctonia solani during a short period of relatively warm, rainy weather.

Botryodiplodia phaseoli

The isolate of Botryodiplodia phaseoli obtained from E. S. Luttrell (Table 1, No. 5) was unique in that it invariably developed pycnospores in the older lesions. The cultural characteristics and the lesions produced on cotton seedlings were identical with those of the other two isolates, which did not produce pycnospores. The pycnidia ranged in diameter from 0.09 to 0.14 mm, with a mean of about 0.1 mm. The sclerotia of the non-pycnidial isolates had a similar appearance microscopically, but were somewhat smaller and ranged from 0.07 to 0.09 mm in diameter. Since the pathogenicity of all three isolates was much the same in the preliminary tests, a mixed inoculum of the isolates, as with other pathogens, was used in the tests.

Table 4. Percent seedling emergence and the number of healthy seedlings per 100 seeds after the number of days indicated for seeds inoculated with Botryodiplodia phaseoli when cultured at 7 temperatures.

Temperature 0°C	Percent seedling emergence	Number of healthy seedlings per 100 seeds after days indicated				
		6	8	10	14	21
18	64				64	54
21	74		74	74	70	60
24	76	76	72	71	65	46
27	80	80	80	78	63	47
30	89	86	84	83	72	63
33	83	80	76	73	68	61
36	83	80	74	60	41	18

Some seedlings were killed at all temperatures, as is shown in Table 4 by the reduction in the number of emerged seedlings. As found in previous studies (46), the fungus killed the seedlings most rapidly at relatively high temperatures; the lowest percentage of surviving seedlings occurred at 36°C. At 30°, 33°, and 36°, the first seedlings were killed after 6 days; at 21°, 24°, and 27° after 14, 7, and 9 days, respectively. At 18°, the first lesion became noticeable after 16 days, and the first dead seedlings were observed 2 days later. At 21°, 24°, and 27°, a few of the seedlings were killed before they emerged from the soil.

Botryodiplodia phaseoli did not always produce distinct lesions on the hypocotyl as did the other seedling pathogens included in this study. The first symptom of infection and injury at 30°, 33°, and 36°C was often the sudden falling over of the seedlings due to the destruction of the interior tissue of the hypocotyl at or near the surface of the soil. This region of the hypocotyl collapsed and became shrunken without the appearance of definite lesions on the surface. A similar effect has been reported for potato stems (57). B. phaseoli was readily isolated from the collapsed hypocotyls. There was no evidence of infection of the roots. As a result there was no evident stunting of the seedlings, even shortly before damping-off, as occurred with the pathogens Colletotrichum gossypii, Pythium ultimum, and Rhizoctonia solani.

In cultures inoculated with B. phaseoli, a large percentage of the seedlings that later showed lesions on the cotyledons did not have corresponding lesions on the hypocotyls. Except for the angular-leaf-spot bacterium, the other pathogens studied produced more abundant lesions on the hypocotyls than on the cotyledons. Cotyledonary infection was usually associated with the adherence of the testas to the cotyledons as they emerged from the soil. The first evidence of such infection was the appearance of small black spots which enlarged rapidly to involve the entire cotyledon. Usually both cotyledons were infected; but occasionally, at 36°C, only one cotyledon became infected. B. phaseoli apparently is not an important cause of losses of cotton seedlings in field plantings, regardless of its demonstrated pathogenicity in this study, since it has been obtained from very few of the diseased seedlings collected by the writer in South Carolina during the past 20 years.

Colletotrichum gossypii

The pathogenicity of Colletotrichum gossypii, the anthracnose fungus, to cotton seedlings is well known from many studies since 1892 (5, 11, 12, 18, 37, 41). Regardless of the relatively detailed information already available on the relation of temperature to infection by this fungus (5, 37), it was included in this study for comparative purposes.

Comparable results were obtained with naturally infested fuzzy seed and with inoculated acid-delinted seed (Table 5). These results approximate those published previously (5) the largest difference being in the greater pathogenicity at 30° and 33°C shown in the later tests. In the previous studies (5), this fungus killed fewer seedlings at 29° than at lower temperatures, and a much smaller proportion were killed at 33°. However, as in this study, lesions appeared sooner at 29° and 33° than at lower temperatures. Whether or not the difference between the cultural methods used in the two studies is sufficient to account for the difference in pathogenicity in the 29°to 30° range is uncertain.

As previously described, the anthracnose lesions first appeared as small, light brown, longitudinal lines (62). Since the lateral extension of the lesions was slow, one cotyledon frequently wilted and collapsed before the other cotyledon was affected. This one-sided character of the lesions produced by the anthracnose fungus usually permits them to be readily distinguished from those due to other pathogens, which generally produce lesions that tend to enlarge laterally as rapidly as they do longitudinally and which, consequently, tend to encircle the hypocotyl at or below the soil surface.

In spite of the large proportion of the seedlings killed at 33°C, typical one-sided lesions were not produced on the surface of the hypocotyl at this temperature. The internal tissues at the base of the hypocotyl were destroyed before definite lesions appeared on the surface. In this respect, the lesions were not greatly different from those produced by Botryodiplodia phaseoli at this temperature.

At 27°C and lower temperatures, the seedlings in the inoculated cultures were decidedly stunted, even in their early growth, as compared with those in the uninoculated. This stunting was only slightly evident at 30° and was not apparent at 33°. At these two temperatures, the seedlings at the time of damping-off were about the size of those in the control cultures. This feature had been noted in the previous studies (5). Lehman (37) found the maximum temperature for this fungus to be 34. 5°C, which explains the failure to infect the seedlings at 36°.

The cultures at 27°C which were planted with acid-delinted seed were the only cultures

Table 5. Percent seedling emergence and the number of healthy seedlings per 100 seeds after the number of days indicated for seed naturally infested by the anthracnose fungus, and also for acid-delinted seed inoculated with the same fungus when cultured at the temperatures indicated.

Temperature 0°C	Percent seedling emergence	Number of healthy seedlings per 100 seeds after days indicated					
		6	8	10	12	14	21
Fuzzy seed - naturally infested							
18	58					40	1
21	83		83	75	55	26	1
24	86	86	77	49	24	1	1
27	91	91	68	23	17	8	1
30	86	80	40	14	11	8	1
33	89	82	79	51	18	15	4
36	91	91	91	91	91	91	91
Acid delinted seed - inoculated							
18	39				58	28	3
21	36	No normal emergence, all dead 11 days					
24	84	84	33	10	4	0	0
27	88	88	85	21	4	0	0
30	86	86	23	0	0	0	0
33	87	87	80	52	46	20	6
36	84	84	84	84	84	84	84

in which a relatively large percentage of the cotyledons were infected by the anthracnose fungus. After 7 days, 30 percent of the seedlings had lesions on the cotyledons. Most of these infected cotyledons were completely rotted 3 to 5 days later.

Fusarium moniliforme

In preliminary tests with Fusarium moniliforme, no differences in pathogenicity were apparent among the six cultures derived from four different host plants (Table 1). Consequently, cultures 5, 6, and 8 were used in the tests from which the results reported with the seed of Coker-100W were obtained. Comparable results were also obtained with seed of the varieties of DPL-15a and Stoneville-2B.

Inoculation of the seeds with this fungus had little or no effect on seedling emergence. Emergence at the several temperatures ranged from 74 to 92 percent (Table 6), essentially the same as for the control cultures (Table 2). There were no evident differences in the size of the seedlings between the uninoculated and inoculated cultures after either the 14- or the 21-day culture period. Consequently, the stunting reported by Woodroof (65) was not noted. Such differences in results with this fungus may be expected, since great variation in pathogenicity seems to be a characteristic of this species (40, 59, 65), and infection of seedlings by it is greatly influenced by environmental conditions (50).

The lesions produced on the hypocotyls by this fungus at 18°, 21°, and 24°C were merely small brown spots on the cortex at or slightly above the collet. None of these lesions became sufficiently extensive to cause typical damping-off; although after 21 days at 18°, 21°, and 24°, the cortex near the base of the hypocotyl was well rotted in 10 percent, 6 percent and 2 percent, respectively, of the seedlings. At 24°, 6 percent of the lesions were the result of cotyledonary infection. F. moniliforme was isolated from them.

Although this fungus produced relatively few lesions and did not stunt the seedlings, the hypocotyls that were plated on agar after 14 days showed relatively large percentages of infection at all temperatures, except 18°C (Table 6). The explanation for the low percentage of seedlings from which the fungus was obtained at 18° is uncertain, since approximately half of the hypocotyls at 21° and 24° were infected after 14 days; and approximately all hypocotyls at

Table 6. Percent seedling emergence for seeds inoculated with Fusarium moniliforme and cultured at the temperatures indicated; and also the precentage of these seedlings from which the fungus was obtained (infected) and the percentage of seedlings that had visible lesions after 14 and 21 days of culture.

Temperature 0°C	Percent seedling emergence	Percentage of infected seedlings		Percentage of seedlings with lesions	
		14 days	21 days	14 days	21 days
18	74	17	22	16	22
21	84	55	100	16	16
24	92	45	88	8[a]	8[a]
27	91	60	16	0	0
30	90	35	18	0	0
33	85	36	43	0	0
36	90	51	72	0	0

[a] 2 percent of these seedlings had lesions on the hypocotyls; the other 6 percent had lesions only on the cotyledons.

these two temperatures were infected after 21 days. In contrast to the results at these two temperatures, after 21 days the percentages of hypocotyls at 27° and 30° from which the fungus was obtained were much smaller than the percentages after 14 days. The explanation for this reduction is again uncertain, unless the cortices of the 21-day-old hypocotyls were penetrated more readily by the hypochlorite solution used for surface sterilization than were those of the 14-day-old seedlings.

This fungus, when it grew from the surfaced-sterilized hypocotyls on water agar, first formed rounded masses of microconidia similar to those illustrated by Voorhees (59, Figs. 2, 8). Later, as the humidity within the dishes became less, the heads were replaced by typical long conidial chains. These frequently had small conidial masses at their bases.

Fusarium moniliforme is one of the more ubiquitous fungi associated with cotton culture in the southeastern portion of the Cotton Belt. It is frequently isolated from superficial reddish-brown lesions on the bases of hypocotyls of seedlings in the field (50). It is almost invariably obtained from the cortex at the base of the hypocotyl as it is being sloughed off with the formation of new cells by the subcortical cambium. The facts that this fungus, when isolated from lesions on the hypocotyl, is often associated with more virulent pathogens, and that it did not cause damping-off in this study, would indicate that it is generally a benign parasite on the cotton plant. One study (26) would indicate otherwise. The data given on the relation of temperature to infection by this fungus would indicate that 21° and 24°C are more favorable for the formation of the lesions on the hypocotyls than are higher temperatures, that 30° is the least favorable temperature for infection, and that after 21 days more seedlings are likely to be infected at 33° and 36° than at 30°.

Fusarium oxysporum f. vasinfectum

Although Fusarium oxysporum f. vasinfectum, the cotton-wilt fungus, is one of the more destructive pathogens of the cotton plant, there are few reports of it as a cause of seedling disease besides those of Elliott (20), Fahmy (22), and Rosen (54). These reports are substantiated by the results given in this paper which indicate that the wilt fungus may cause damping-off of cotton seedlings at relatively low temperatures. The data reported were derived from the inoculation of seeds of Coker-100W, a variety considered to be generally resistant to the wilt disease. The isolate of the fungus (Table 1) was obtained from a field of Coker-100W in which plants showed wilt symptoms.

The emergence of the seeds inoculated with the wilt fungus was about the same as that for uninoculated seeds (Tables 2 and 7), except for a smaller emergence at 21°C. Inoculation with this fungus produced most noticeable stunting of the infected seedlings at 18° and 21°. At the higher temperatures, the small light tan lesions did not have any apparent effect on seedling growth; and there was little stunting of the seedlings until the lesions involved the entire circumference of the hypocotyl, except for those seedlings with infected cotyledons at 33° and 36° (Table 7). Although the fungus was recovered from 13 percent of the hypocotyls at 36° after

Table 7. Percent seedling emergence for seed inoculated with Fusarium oxysporum f. vasinfectum and cultured at the temperatures indicated; and also the percentage of these seedlings from which the fungus was obtained (seedlings infected), the total percentage of seedlings with lesions, the percentage of the seedlings with extensive lesions, and the percentage of the seedlings killed after 14 and 21 days of culture.

Temperature 0°C	Percent seedling emergence	Percentage of seedlings after indicated number of days							
		Infected		With lesions		With extensive lesions[a]		Killed	
		14 days	21 days	14 days	21 days	14 days	21 days	14 days	21 days
18	65	41	48	15	68	0	50	0	50
21	74	75	100	10	42	10	40	8	40
24	86	75	97	14	48	12	14	0	40
30	90	32	12	3	3	3	3	0	3
33	84	20	55	3[b]	41	3[b]	15	0	0
36	90	21	13	1[b]	1[b]	1[b]	1[b]	0	1

[a] Lesions which were extensive enough to preclude normal seedling growth.
[b] Lesions on cotyledons only.

21 days (Table 7) no lesions were produced on the hypocotyls at this temperature.

The hypocotyl lesions associated with infection first appeared as light tan, somewhat circular areas, 1 to 2 mm in diameter, that enlarged slowly. Initially they were confined to one side of the hypocotyl. Later they became dark brown in color and even blackish, coincidental with their invasion by bacteria. In some instances, the lesions increased in size and finally involved the entire circumference of the hypocotyl below the soil level. When this occurred, especially at the higher temperatures, 27° to 33°C, the infected area lost its turgidity and the seedlings showed the typical symptoms of damping-off. After 14 days, the highest percentage of seedlings (12 percent) with such lesions was at 24° (Table 7). After 21 days, these percentages at 18°, 21°, 24°, 27°, 30°, and 33° were 50, 40, 14, 6, 3, and 15, respectively. At 18° and 21°, these infected seedlings were dead. At the higher temperatures, 24°, 27°, and 30°, most seedlings with extensive lesions were still alive after 21 days, but it is doubtful whether they would have survived under field conditions. The only temperature at which the seedlings were killed (8 percent) before the 14th day was at 21° (Table 7). At 33° and 36° after 14 days a small percentage of the seedlings had their cotyledons destroyed by the fungus (Table 7).

When the seedlings were plated to ascertain the percentages infected, it was found that the percentages infected were much larger than the percentages with lesions, except at 18°C after 21 days. The differences were especially large at 21° and 24° after 14 and 21 days, and at 27° and 33° after 21 days. At 27° after 21 days, 24 seedlings with and without lesions were plated. The fungus was obtained from half of both groups of seedlings. It is apparent that this fungus may invade the tissues of a cotton seedling without causing visible lesions. The fungus obtained must also have been the same as that used to inoculate the seeds, since a similar fungus was not obtained from uninoculated cultures grown concurrently.

The data for the 21-day growth period indicate that the least favorable temperatures for the infection of the hypocotyl were 30° and 36°C; for the development of lesions, 30° and 36°; and for the production of severe lesions, 27°, 30°, and 36°. At 33° after 21 days higher percentages were infected and had lesions than at 30°. In this respect the results at 33° are more nearly comparable with those at 24° and 27° than at 30°. Thus, the cotton seedling appeared to have a maximal resistance to infection at 30°, and its susceptibility to infection and injury increased at lower and at higher temperatures.

The data in Table 7 show three anomalies which are difficult to explain and which did not appear to a comparable extent in all tests with this fungus. They are 1) the failure to obtain a fungus from all seedlings with lesions at 18°C after 21 days, and 2) the lower percentages of infected seedlings at 30° and 36° after 21 days than after 14 days. As indicated previously, in the Fusarium moniliforme cultures also a lower percentage were infected after 21 days than after 14 days at 27° and 30°.

Similar results were obtained in another test with a Fusarium-wilt isolate, in which the seedlings were grown in controlled temperature tanks in the glasshouse and at soil tempera-

tures of 18°, 21°, 24°, 27°, and 30°C. The mean glasshouse temperature was about 25°, with a daily range of 15° to 30°. The test was terminated after 3 weeks when the check plants at 27° had a mean height of 45 cm. The percentages of plants with lesions at or just below the surface of the soil were much the same as in the laboratory tests, being 100, 30, 35, 41, 2, and 2, respectively, for 18°, 21°, 24°, 27°, 30°, and 33°. All but 3 percent of the seedlings were killed at 18°. The only plants to show discoloration of the stele were those grown at 27°, 30°, and 33°.

As previously described by Rosen (54), the color and nature of lesions produced by the Fusarium oxysporum isolates may not be greatly different from those produced by Rhizoctonia solani, except that in these studies they enlarged less rapidly than Rhizoctonia lesions and were mostly confined to one side of the hypocotyl. The superficial lesions at first were a light tan in color, gradually becoming deeper and browner. Later, coincidental with invasion by bacteria, they became dark and black.

Since it has been shown that isolates of Fusarium oxysporum from the sweetpotato, Ipomoea batatas, may infect cotton without necessarily producing symptoms of wilt (3), an isolate highly pathogenic to sweetpotato was compared at 21°C with that from cotton. The percentage of emerged seedlings for the sweetpotato and cotton isolates were 82 and 74, respectively. The differences in the percentages of seedlings from which the respective fungi were obtained were slightly greater. Thus, after 14 days the percentage for the sweetpotato isolate was 50, for the cotton isolate 75; after 21 days, the percentages were 87 and 100 respectively. The sweetpotato isolate, however, produced fewer lesions on the hypocotyls than the cotton isolate. The former had produced lesions on only 2 percent of the hypocotyls after 14 days and no additional lesions appeared. In contrast, the cotton isolate had produced lesions on 10 percent of the hypocotyls after 14 days, and 42 percent after 21 days. Thus, the two isolates were not very different in respect to infection of hypocotyls, but were greatly different in respect to capacity to produce lesions.

Over a period of 20 years in which the writer had examined and plated large numbers of seedlings, Fusarium oxysporum f. vasinfectum was found definitely associated with seedling losses in only one field. These losses occurred after a period of relatively heavy rainfall and low temperatures. This field was known to have a high inoculum potential for this fungus. The seedlings examined were also abundantly infested by several species of nematodes.

The data which indicate a greater infection of cotton seedlings by the wilt fungus at relatively low temperatures, 18° to 24°C, compare with those obtained by Porter and Melhus (47) and Walker (60) for Citrullus spp. Other studies with cotton and other plants agree that the optimum temperature for the expression of wilt symptoms is 27° or slightly higher (15, 20, 47, 66). Similarly, during the studies reported in this paper, the dark discoloration of the stele that is typical of wilt symptoms was observed only at the temperatures of 27°, 30°, and 33°, but not at lower temperatures; thus, it would appear that the optimum temperature for infection by the wilt-producing Fusaria may be lower than that for the appearance of internal discoloration.

A culture of Fusarium solani (Mart.) Appel & Wr. obtained from a diseased cotton seedling in a field in which a large proportion of the seedlings were dying as the result of infection by various pathogens was used in a similar study. At soil temperatures of 20° and 25°C, it produced larger lesions and more extensive damping-off than the wilt isolates. The cultural conditions were such that the results cannot be compared with those reported in this paper; but the results supplied unquestionable proof that F. solani could cause extensive damping-off at a soil temperature of 20°, and at 25° could produce lesions on the hypocotyl and cause discoloration of the stele in a small proportion of the seedlings. Fusarium roseum (Lk.) emend. Snyd. & Hans. has also been obtained from infected cotton seedlings (10).

Pythium ultimum

Published data on Pythium ultimum as a parasite of cotton seedlings (4) indicate that it is a highly virulent pathogen at soil temperatures of 18°, 21°, 24°, and 27°C. Few seedlings were infected at 30°, and seedlings grown at this temperature for 12 days and then grown at a lower soil temperature were no longer severely injured. In the present study, in which both air and soil temperatures were approximately the same, no seedlings emerged at 18° and 21°; this demonstrates a more rapid destruction of the seeds and seedlings by this pathogen than by any of the others studied. At 24° (Table 8) emergence was only 34 percent, and the number of seedlings gradually decreased until only 23 percent were alive after 14 days, and 21 percent after 21 days. The first seedlings damped-off on the eighth day. The 23 seedlings alive after

Table 8. Percent seedling emergence for seeds inoculated with Pythium ultimum and cultured at the temperatures indicated, and also the number of surviving seedlings per 100 seeds after 14 and 21 days of culture.

Days	Temperature 0°C						
	18	21	24	27	30	33	36
Percent seedling emergence							
21	0	0	34	71	90	88	88
Surviving seedlings per 100 seeds							
14	0	0	23[a]	52	90	88	88
21	0	0	21[b]	47	90	88	88

[a] All stunted.

[b] Only one seedling was without a lesion; the other 20 were infected by P. ultimum, and the seedlings were 25 to 40 percent the size of those in controls.

14 days were only 60 percent to 80 percent as large as those in checks. Twenty of these were alive after 21 days, and only one of these seedlings was of approximately normal size. The others were 25 to 40 percent as large as the checks. P. ultimum was isolated from all the seedlings with lesions.

At 27°C, the percentage of emerged seedlings was 19 percent less than in the controls; the first seedling damped-off on the eighth day; and there was a further reduction to 52 percent of living seedlings after 14 days. A further reduction of 5 percent occurred before the 21st day. Of the 24 seedlings removed after 14 days, 18 were not infected and 6 had lesions on the hypocotyls from which the pathogen was recovered. Four of the surviving seedlings removed after 21 days had relatively large lesions on the hypocotyls from which the fungus was recovered. About 10 percent of the seedlings had small 1-2 mm lesions on cotyledons, but no fungi were obtained when they were plated. At 30°, 33°, and 36°, no seedlings were infected. Harter and Whitney (27) found a somewhat similar relation of temperature to the decay of sweet-potatoes when infected by this fungus, although their data indicate a growth optimum for the fungus at 32°. The latter is somewhat higher than the optimum found by Halpin et al. (25).

The nature of the injuries to the hypocotyl and roots of the cotton plant is very similar to that of injuries produced on red clover seedlings by P. ultimum and other Pythium spp. (25). Thus, after the infection of cotton seedlings by P. ultimum, the cortex of the root or hypocotyl appears as if infiltrated with water and the cortical tissues quickly collapse. The lesions tend to remain tan or light brown in color until invaded by other microorganisms, when they change to dark brown or black. The initial lesions generally appeared on the hypocotyl just above the collet. However, lesions were often present on the tap and secondary roots. If infection of the hypocotyl occurred 2 to 3 weeks after emergence, only small cortical lesions were formed. Most of the seedlings that emerged at 24° and 27°C formed secondary roots and the latter were also infected, as was indicated by small, dark lesions. This infection of the roots apparently accounted for the noticeable stunting of the surviving seedlings after 21 days at 24° and 27°.

P. ultimum causes a relatively small proportion of the losses of cotton seedlings in the field (62), even though it is a virulent pathogen. It is usually very destructive in small, sharply delineated areas in which few seedlings may survive. The erratic distribution of such areas makes it difficult to incriminate any particular soil condition as a predisposing factor to seedling infection, except that infections are invariably associated with periods of relatively high soil moisture. It is also possible that this fungus may have been present in more instances of seedling losses than reports would indicate, since on non-nutrient agar it forms relatively inconspicuous mycelium which may be readily overlooked because of the usual presence of fungi with more conspicuous growth.

Rhizoctonia solani

Rhizoctonia solani is generally considered to be the cause of greater losses of cotton seedlings than any other pathogen when likelihood of infection by the anthracnose fungus is elimi-

nated by seed treatment. This is largely due to its widespread geographical distribution and the severity of the sporadic losses of stand which occur when conditions are favorable for seedling infection. The fact that seed treatments are only partially effective in preventing seedling infection by R. solani (38) is one of the reasons for the intensive study of its parasitism on the seedlings. It was described by Atkinson in 1892 (11) as a pathogen which causes damping-off of cotton. A study of the temperature relations of its parasitism on cotton was made by Walker (60), who also reviewed the pertinent, available literature.

As indicated in Table 1, three isolates from cotton seedlings were used in these tests. In the preliminary tests, three additional isolates were found to be equally pathogenic at 24°C. They were supplied by W. W. Ray, who isolated them from snap bean (Idaho), soybean (Oklahoma), and cotton boll (Oklahoma). Culture No. 23, Table 1, invariably formed basidiospores when infected seedlings were plated on non-nutrient agar. The length of sterigmata approximated that described for flax strains (26 microns) by Vanterpool (58). Basidiospores were not found on seedlings from cultures inoculated with the other two isolates. All isolates tested were similar in cultural characteristics to type A of Houston (31).

Table 9. Percent seedling emergence for seeds inoculated with Rhizoctonia solani and cultured at the temperatures indicated, and also the number of surviving seedlings per 100 seeds after 14 and 21 days of culture.

	Temperature 0°C						
Days	18	21	24	27	30	33	36
Percent seedling emergence							
21	25	5	65	78	69	81	83
Surviving seedlings per 100 seeds							
14	0	0	5	13	30	49	86
21	0	0	0	0	16	19	86

This fungus was most destructive at 21°C, at which temperature only 5 seedlings emerged (Table 9); and lesions were visible on the hypocotyls when they emerged. All of these seedlings had died before the 14th day. At 18° it was only slightly less virulent, and after 11 days there were 25 percent emerged seedlings. None of these survived to the 14th day. At 24°, 27°, 30°, and 33°, the percentage of emergence varied from 65 to 81. The emerged seedlings, however, quickly succumbed to infection and after 14 days the number of seedlings was greatly reduced. At the several temperatures, 18°, 21°, 24°, 27°, 30°, and 33°, the first seedlings were killed after 11, 10, 7, 5, 6, and 6 days, respectively. At 30° and 33° after 21 days, the living seedlings were stunted and had small lesions which had destroyed most of the cortex over a small area below the soil surface; but they were then forming new roots below the lesion and would likely have survived. These were the only temperatures at which all infected seedlings were not killed.

The first symptom of infection of the hypocotyl was the appearance of a small, light brown or tan lesion on its base, which enlarged rapidly to girdle the hypocotyl. This was accompanied by a soft rot and disintegration of the cells in the affected area. Seedlings were killed within 48 hours of the appearance of the initial lesion. The cotyledons frequently did not wilt until the seedlings had damped-off. This fungus may also infect and kill the roots (9). This type of infection resulted in the stunting of the seedlings, as was evident in the 18° to 27°C range. After 11 days the seedlings in the inoculated cultures at 27° were only 1/3 as large as those in the check cultures.

The cortex of seedlings is sometimes infected above the soil level. In such cases conspicuous sunken, dark brown lesions with sharply delineated margins may be formed. Such lesions may enlarge and girdle the stem. This fungus may also infect the foliage of cotton plants (44).

Infection of the seedlings by this pathogen is generally described as being associated with relatively cool, rainy weather; but in this study it was still an active parasite at 30° and 33°C,

which temperatures are close to and slightly above the optimum for growth of typical isolates (13). Walker (60) obtained comparable results with cotton seedlings in the 19° to 34.5° soil temperature range. Its virulence over a wide range of temperatures would seem to indicate that it should be almost impossible to obtain a stand of cotton seedlings in fields in which this fungus is well distributed, except that its parasitism in the field is greatly influenced by environmental factors other than temperature (13, 21, 33, 36, 51, 52, 55).

Thielaviopsis basicola

The three isolates of Thielaviopsis basicola used (Table 1) were obtained from two hosts, cotton and tobacco, and from three geographical locations, Mississippi, North Carolina, and Canada. Preliminary tests at 24°C showed no apparent differences in pathogenicity among them. Consequently, the three isolates were combined for the inoculation of the cultures on which the accompanying data are based.

Table 10. Percent emerged seedlings for seeds inoculated with Thielaviopsis basicola and cultured at the temperatures indicated; and also the day of culture on which the first seedling was killed by the fungus, and the number of living and normal seedlings per 100 seeds after 14 and 21 days of culture.

Temperature 0°C	Percent seedling emergence	Day on which first seedling was killed	Seedlings per 100 seeds after 14 days		Seedlings per 100 seeds after 21 days	
			Alive	Normal	Alive	Normal
18	64	18	64	23	27	0
21	85	10	49	0	31	0
24	88	14	87	2	52	0
27	92	18	92	3	55	0
30	95	14	95	3	55	2
33	92		92	92	92	92

This fungus infected the seedlings through a temperature range of 18° to 30°C (Table 10). After 14 days, the lowest percentage of surviving seedlings was at 21° (49 percent). The taproot, or hypocotyl, of essentially every seedling was infected through the range of 21° to 30°. The lesions appeared somewhat later at 18° than at 21°, but after 21 days practically all seedlings at this temperature were also infected. An unique feature of infection by this fungus was the sharp delineation of the temperature at which seedlings were infected. As indicated above, the fungus was a virulent parasite at 30°, yet at 33° no lesions were produced. This observation agrees with that of Johnson and Hartman (32), who found that the upper limit for infection of tobacco was below 32°. Most of the other pathogens used in this test showed a more gradual change in the percentage of infected seedlings as the temperature at which the plants were grown was raised.

In these studies the first evidence of infection was the falling over of the seedlings -- the hypocotyl bending sharply at the surface of the sand. The cotyledons of the prostrate seedlings generally remained unwilted for several days. Lesions appeared initially on the proximal portion of the taproot immediately adjacent to the original position of the seed, and such lesions constituted most of those that were present on the seedlings after 14 days. Necrosis of the hypocotyl became evident only after the cortex of the taproot was well blackened and necrotic. The failure of the prostrate seedlings to wilt appeared to be associated with the decay of the cortex before the stele was seriously affected. Frequently, the cortex of the proximal part of the taproot was completely rotted and sloughed off; yet the stele was normal in color and the seedling was unwilted and was making some growth. Death of the seedlings was usually associated with discoloration of the stele. If this did not occur before the seedlings reached a certain size -- equivalent to growth for 3 weeks at 27°C -- the seedlings formed new roots both above and below the lesion and seemed capable of forming normal plants. These observations are somewhat at variance with those reported for older plants by King and Presley (34), whose description of the disease would indicate that the stele is blackened before there is extensive

damage to other tissues.

After 3 weeks at 18°, 21°, and 24°C, it was still uncertain as to what percentage of the surviving seedlings would have been able to make further growth. The tops were mostly prostrate, but the cotyledons had not wilted. If these seedlings had recovered, they might have remained stunted as suggested by Blank (14). In contrast to the severe injury to the seedlings in these tests after 21 days at 18° to 30°, Blank (14) states that the first above-the-ground symptoms appeared after 4 to 5 weeks of culture. Whether or not the difference in the time required for the symptoms of infection to appear in these two studies is explicable on the basis of a difference between the varieties of cotton or the techniques used is uncertain.

One of the characteristic features of infection by this fungus was an evident stunting of plants long before they became prostrate or before lesions were apparent on the hypocotyl above the surface of the soil. Weights of the surviving seedlings were recorded for the several temperatures after 21 days; at this time the mean seedling weights in the inoculated cultures relative to the controls were: 18°C, 90 percent; 24°, 75 percent; and 27°, 84 percent. At 30° there was no evident stunting and about 50 percent of the surviving plants were forming abundant secondary roots. This fungus did not produce lesions on the cotyledons.

As indicated by previous papers (14, 34), this fungus has rarely been reported as a cause of seedling losses in cotton. It is not listed as a fungus infecting cotton seedlings in the Southeast (50, 62), nor has it been isolated by the writer. The high pathogenicity herein reported and the effects noted previously (14, 34) indicate that it may be a destructive pathogen when present in the soil. Thus, these data would seem to indicate that cotton plants would be likely to be infected by this fungus if grown in a field in which black root-rot of tobacco had been prevalent..

Verticillium albo-atrum

Since Presley (48) discussed the distribution of the Verticillium wilt of cotton, there has been one report of a seasonal, but widespread occurrence of this disease in North Carolina (39). Presley (48) states that seedling infection by Verticillium albo-atrum results in yellowish cotyledons, desiccation, and usually the death of the seedling. Otherwise, there is little in the literature to indicate that this fungus may be the cause of a disease of cotton seedlings.

Three isolates from cotton (Table 1) were used in the tests. The germination percentages in the inoculated cultures were the same as in the checks. No seedlings showed infection at any temperature after 14 days. After 21 days at 24°C and at higher temperatures, there were no lesions on the hypocotyls or roots, while at 18° and 21° there were small black lesions, 1 to 3 mm in diameter, on 6 percent and 2 percent respectively of the hypocotyls. No fungi were obtained when these hypocotyls were surface sterilized and plated.

Since the parasitism of the fungus on the cotton plant is well substantiated, other cultures were inoculated with the same three isolates and grown in a glasshouse with a maximal daily range in temperature of 15°.to 28°C. The first wilted seedlings were observed after 24 days in cultures that had been adjusted to pH 7.5 by the addition of $CaCO_3$. Yellowish-brown areas, 1 to 3 mm in diameter, were present on the hypocotyls of the wilted seedlings. The adjacent stele was discolored for 1 to 2 cm beyond the lesion. The fungus was recovered from these hypocotyls. No infection was observed in the more acid cultures. These observations indicate that a somewhat longer culture period than 21 days is needed to produce appreciable injury to the seedlings, and also that the parasitism of this fungus, as previously reported (24), is greatly influenced by soil conditions.

Xanthomonas malvacearum

Xanthomonas malvacearum is recognized as a common cause of seedling blight on susceptible varieties of cotton (23, 42, 53) when seed either infested and/or infected by this bacterium are planted. Massey (42) has indicated a temperature range for severe infection of 21° to 28°C, with no infection at 28° and higher.

Two cultures (Table 1), derived from infected plants in Texas and Oklahoma, were used in the study. Cultures on raisin-oats agar were used to make bacterial suspensions in which the seed were immersed for 4 hours. No infection was obtained when seed of the Stoneville-2B and Coker-100W varieties were thus inoculated. A lot of seed of the susceptible variety Acala was then obtained and used for the studies reported here. In preliminary tests, the lesions produced were confined to the cotyledons. No hypocotyls were infected. Consequently, an attempt was made to maintain the cotyledons at a given temperature. To accomplish this the air

temperature was lowered immediately after cotyledonary emergence so that the cotyledons would be subjected to the same temperature as before emergence. As indicated previously, this involved reducing the air temperature from 37.5° to 36°C at the highest temperature, from 18.5° to 18° at the lowest temperature, and intermediate amounts at the other temperatures.

Lesions were not produced on the hypocotyls at any of the temperatures. The first lesions on the cotyledons became evident in from 5 to 10 days at 21°C and higher temperatures (Table 11). No lesions were produced at 18°. The number of cotyledons with lesions was less at 21° than through the higher temperature range of 24° to 36°, and there was little difference after 14 days in the number of lesions and the severity of the injury to the cotyledons in this temperature range. At 36°, the lesions were mostly confined to the margin of the cotyledons.

Table 11. Percent seedling emergence for Acala cotton seed inoculated with Xanthomonas malvacearum and cultured at the temperatures indicated; and also the day of culture on which lesions were first observed on the cotyledons, and the number of seedlings per 100 seeds on which cotyledonary lesions were present after 14 days of culture.

Temperature 0°C	Percent seedling emergence	Days of culture to appearance of first lesion	Seedlings per 100 seeds with cotyledonary lesions on 14th day of culture
18	63	0	0
21	86	10	25
24	86	8	44
27	84	7	43
30	81	5	36
33	76	5	33
36	83	6	50

Except at 30° and 33°C after 14 days, there was no further increase in the number of lesions and little increase in the degree of injury to the cotyledons. At 30° the cotyledons of 26 of the seedlings (100-seed basis) that did not have infected cotyledons after 14 days had extensive cotyledonary lesions after 21 days. The corresponding number at 33° was 20. Consequently, the total numbers of seedlings with infected cotyledons at these two temperatures after 21 days were 62 and 53, respectively. In view of the conditions necessary for infection (64), there is no certain explanation for the increase in the number of lesions after 14 days.

After 21 days, the stunting effect of cotyledonary infection was more noticeable in the 24° to 33°C range than at 21° and 36°. At 33°, the mean weight of the seedlings in the inoculated cultures was only 77 percent of the weight of seedlings in the uninoculated cultures. At 30° the stunting was only slightly less, and the relative weight of the seedlings in the inoculated cultures was 83 percent. At this temperature, the lesions were slightly more numerous than at 33°, but the lesions tended to be somewhat smaller. At 21° there was no evident stunting and the largest lesions were not greater than 2 mm in diameter. Through the range 24° to 33°, some cotyledons were completely destroyed. It is rather difficult to reconcile these results with Massey's statement (42) to the effect that little infection is to be expected above 28°C.

It should be evident from the small effect that infection of cotton seedling cotyledons by X. malvacearum does not result in typical damping-off. Under certain conditions, however, the injury to cotyledons can be sufficiently severe to stunt and to retard the growth of seedlings, and thus predispose seedlings to infection and injury by the more virulent soil-inhabiting pathogens.

DISCUSSION

The first protective structure of the taproot and lower hypocotyl, the epidermis, is not highly effective as a barrier to infection by fungi. This epidermal barrier becomes ineffective when the epidermis and cortex are ruptured by the development of a sub-endodermal cambium and periderm in the initiation of secondary growth and by the development of secondary roots.

The periderm develops irregularly, and it may not form a complete, protective cylinder for some time after the cortex is ruptured. It is at this phase of development that the hypocotyl is especially susceptible to invasion by pathogens and nematodes; and any environmental condition which delays the formation of a complete periderm greatly predisposes the seedling to infection by fungi and invasion by nematodes. Consequently, this is a very critical period in the life cycle of the cotton seedling; and its survival and future normal growth are often dependent on the rapid development of a complete periderm after the epidermis has been ruptured. The cortex, after initiation of secondary growth, assumes a yellow to yellowish-brown color which closely simulates the color of the lesions produced by certain fungi. Fusarium spp., bacteria, and nematodes are usually present in the cells of the cortex at this time. After the periderm has become well developed, usually in 2 to 4 weeks, the hypocotyl region is no longer susceptible to infection by some pathogens. This applies especially to Colletotrichum gossypii and Pythium ultimum, and to a lesser degree to Rhizoctonia solani.

Table 12. The pathogens infecting cotton seedlings, the temperature range through which they infect seedlings, and primary source of inoculum.

Pathogen	Temperature range (°C)		Primary source of inoculum in field
	For infection	For severe damage	
Ascochyta gossypii	18°-27°	18°-27°	Seed, hosts other than cotton
Botryodiplodia phaseoli	18°-36°	36°	Soil[a]
Colletotrichum gossypii	18°-33°	18°-33°	Seed, soil occasionally
Fusarium moniliforme	18°-36°	None	Seed and soil
F. oxysporum f. vasinfectum	18°-36°	18°-24°	Soil, seed rarely[b]
Pythium ultimum	18°-27°	18°-24°	Soil
Rhizoctonia solani	18°-33°	18°-33°	Soil, seed occasionally
Thielaviopsis basicola	18°-30°	18°-21°	Soil
Verticillium albo-atrum	18°-21°	?	Soil, seed rarely
Xanthomonas malvacearum	21°-36° [c]	24°-36° [c]	Seed and soil

[a] The term soil is used to include inoculum which may have survived on plants and plant debris on or in the soil.

[b] Elliott (19) has shown that this fungus may be carried in a small percentage of the seeds derived from infected plants. Other Fusarium spp. have been frequently isolated from cotton seeds. The writer, however, has germinated numerous seeds produced by severely wilted plants without finding a single seed that was infected by the wilt fungus.

[c] On cotyledons only.

In these studies most of the pathogens were destructive over a wider temperature range than they are ordinarily under field conditions (Table 12). The innoculum potential should probably be placed first among the factors that affected pathogenicity, since relatively large amounts of the mycelium of the pathogens were placed on the seeds. The soil moisture conditions and the relatively high humidity also should have been favorable for a high incidence of infection. In these tests the pathogen under study also had little competition with other fungi, since autoclaved sand was used as the culture medium. Under field conditions, there is competition among the numerous soil microorganisms, some of which are antagonists of the pathogens (1, 52, 63).

The mixed-isolate cultures of the several pathogens used in this study may also have extended the range of pathogenicity of a given species somewhat further than that which would have been obtained for a single isolate. However, the studies carried out initially with the separate isolates at several temperatures did not show significant differences in pathogenicity among the isolates of a given species. Since the primary objective of this study was to ascertain the range of temperature over which a given pathogen would infect cotton seedlings and to obtain information on the nature of lesions produced, the use of mixed cultures appears to have been the most convenient method of obtaining this information.

Since the data in Table 12 show that the pathogenicity of the pathogens must be influenced by soil temperature, a résumé of soil temperatures after the usual time that cotton seed is planted should indicate the manner in which these temperatures influence the incidence of the diseases of the cotton seedling. Soil temperatures taken in South Carolina over a period of years (16, Fig. 5) show that the maximum daily soil temperatures after cotton has been planted may frequently exceed 35°C on clear days. Temperatures as high as 42° have been recorded within 3 weeks after planting. On cloudy days and those with rainfall, soil temperatures are lower and approximate temperature of the air. Thus, for some days during rainy weather, the maximum daily temperature may not exceed 20° (16, Fig. 5, A). As soon as the soil loses its high moisture content the soil temperature at 5 cm may exceed that of the air temperature by 5° to 10°. Nearer the soil surface the temperatures must be even higher. Although these high temperatures may not kill the pathogens, they are undoubtedly high enough to inhibit their growth temporarily; and the capacity of the pathogens to injure the seedlings severely should be reduced in the following order as the temperature becomes higher: Verticillium albo-atrum, Thielaviopsis basicola, Fusarium oxysporum f. vasinfectum, Ascochyta gossypii, Colletotrichum gossypii, Rhizoctonia solani, Botryodiplodia phaseoli, and Xanthomonas malvacearum.

SUMMARY

Ascochyta gossypii is not known to be a frequent cause of the damping-off of cotton seedlings, regardless of its proven pathogenicity and the number of other hosts. However, infection of the cotyledons and leaves after seedling emergence may retard seedling growth and predispose the seedlings to infection and severe injury by soil-inhabiting pathogens. Infections of the hypocotyl at or below the soil level by this fungus in these tests resulted in the formation of reddish-brown to blackish lesions which enlarged rapidly to involve the entire circumference of the hypocotyl. Several days later the seedlings broke over at the soil level, but did not wilt until after several more days. The stele was not destroyed until after the cortex was well rotted. This fungus may infect and kill seedlings at 27°C and lower temperatures. Infection did not occur at 30°.

Botryodiplodia phaseoli was the only fungus tested which showed a high pathogenicity at 36°C. It was weakly parasitic at lower temperatures as compared with Colletotrichum gossypii and Rhizoctonia solani. At 30°, 33°, and 36°, when infection did occur, the fungus often invaded the internal tissues at the soil level and caused the collapse of the hypocotyl before there were external symptoms of infection. A small percentage of the cotyledons were also infected at these temperatures. In some instances, small dark lesions just below the surface of the soil enlarged to involve the entire cortex and stele. This fungus has been isolated only occasionally from diseased seedlings.

Colletotrichum gossypii -- The typical lesion that results from infection by this fungus is marked by the appearance on the base of the hypocotyl of a longitudinally elongated tan area, which becomes darker brown and forms a depressed area in the cortex. A distinguishing characteristic of the lesions produced by this fungus is that they enlarge more rapidly longitudinally than transversely. Consequently, one side of a hypocotyl may be rotted and its corresponding cotyledon wilted, while the opposite cotyledon remains turgid for several days longer. Cotyledons may be infected at 27°C and lower temperatures. These infections first result in small black spots which under appropriate temperature and humidity conditions may enlarge to involve the entire cotyledon. In contrast to most of the other pathogens studied, this fungus does not invade the taproot until after the hypocotyl has been severely injured.

Since this fungus is usually dispersed by mycelium carried in or on the seed (internally infected seed rarely form seedlings), the infection is generally uniform throughout the field when untreated infested seed are planted. Seedling losses are generally greatest on sandy soils. This is not related to the degree of infection, but to the rapidity with which the soils dry out after relatively high soil moisture has predisposed the seedlings to infection. The enlargement of lesions at the base of the hypocotyl may be inhibited by hot, dry weather. When this occurs, the seedlings may develop new secondary roots, frequently on the hypocotyl. Whether or not the seedling survives depends upon the capacity of the injured hypocotyl to supply sufficient water to the seedling until the new roots are developed. The hypocotyl is not susceptible to infection by this fungus after a continuous cylinder of periderm has been formed. This fungus killed the seedlings most quickly at 27° and 30°C, but there were few surviv-

ing seedlings in these studies in the range of 18° to 33°.

Fusarium moniliforme, when isolated from lesions on cotton hypocotyls, is usually associated with more aggressive parasites. The results reported indicate that it may produce lesions on a small percentage of cotyledons and hypocotyls at 18° to 24°C. It is also often the only fungus isolated from reddish-brown superficial cortical lesions below the soil surface at about the time that secondary growth is initiated. Probably the most striking feature of the results with F. moniliforme was the large proportion of the seedlings from which the fungus was recovered but which had no apparent lesions. Further study will be necessary to ascertain whether the fungus had penetrated beyond the cortex. All results with this species must be interpreted on the basis of its known variation in pathogenicity.

Fusarium oxysporum f. vasinfectum produced lesions on the hypocotyls of cotton seedlings through a temperature range of 18° to 33°C. Cotyledons were also infected. The lesions produced on the hypocotyl were not greatly different initially from those produced by Rhizoctonia solani, except that they enlarged less rapidly, tended to be confined to one side of the stem, and finally became blackish rather than reddish-brown. Only at 18° and 21° did these lesions become extensive enough to kill the seedlings; and after a 21-day period of culture at temperatures of 21° and higher, the number of seedlings from which the fungus was reisolated was much greater than the number with lesions. Other Fusarium species, as F. solani, may produce similar lesions on the hypocotyl. The Fusarium spp. were aggressive pathogens only at relatively low temperatures, 21° and lower. Thus, the temperature that is favorable for seedling infection by Fusarium oxysporum f. vasinfectum is lower than that which is favorable for the expression of wilt symptoms in older plants.

Pythium ultimum -- The lesions produced on the base of the hypocotyls and the roots of cotton seedlings by Pythium ultimum initially are not greatly different from those resulting from infection by Rhizoctonia solani. P. ultimum tends to initiate a soft, watery rot of the cortex of the roots and hypocotyl that remains light-brown in color until the lesion is invaded by other fungi. These lesions rarely assume the reddish-brown characteristic of older R. solani lesions. As with the latter fungus, the lesions tend to girdle the hypocotyl rather than to extend longitudinally. P. ultimum does not infect the aerial parts of the plant. Infection in the field is usually associated with several days of rainy weather, and the data given indicate that it is an aggressive parasite only at relatively low temperatures. The maximum temperature for infection is approximately 27°C.

The geographic distribution of the areas in which seedlings are infected is even more erratic than for R. solani. The areas are generally relatively small, and the disease has a tendency to appear each year in the same areas when they are planted to cotton. Thus, infection by this pathogen, because of its association with certain limited soil areas, causes much less extensive losses than R. solani.

Rhizoctonia solani -- In contrast to the usual uniform incidence throughout the field of the anthracnose disease, infection by this fungus is likely to be more severe in certain areas of a field, usually those with heavier soil or more poorly drained. All parts of the seedling may by infected -- root, hypocotyl, and cotyledon. Usually the initial infection occurs just below the level of the soil. Under favorable conditions for the fungus, the infected, light brown, water-soaked area will rapidly enlarge to encircle the hypocotyl and kill the seedling in 1 to 2 days. The enlargement of the lesion may be stopped quickly by dry, warm weather. If infection of the cortex occurs after secondary growth has been initiated, the lesions are generally confined to the cortex. In these instances, a reddish-brown to dark-brown sunken area is produced. R. solani killed most of the seedlings through the range of 18° to 33°. No seedlings were infected at 36°C.

Thielaviopsis basicola -- The initial lesions that are produced by this fungus enlarge more slowly than those produced by Colletotrichum gossypii, Rhizoctonia solani, or Pythium ultimum. Roots and hypocotyls are about equally susceptible, although in these studies the lesions first appeared on the upper portion of the taproot. The cortex may be completely destroyed before the stele is sufficiently affected to result in wilting of the seedling. The blackish color of the lesions produced by this fungus is another diagnostic feature. The writer has not observed this fungus as a cause of the damping-off of cotton seedlings in the field. This fungus was an active parasite through the range 18° to 30°C; no seedlings were infected at 33°.

Verticillium albo-atrum -- Although this fungus is a well known parasite of the cotton plant, the three isolates of the fungus used in this study did not produce lesions on the seedlings at 24°C and higher temperatures; and at lower temperatures produced small lesions on only a small percentage of the seedlings. Infection did occur and wilted seedlings were obtained when cultures adjusted to pH 7.5 were grown in a greenhouse for 24 days. Small black lesions on the surface of the hypocotyls were associated with a more extensive discoloration of the stele.

Xanthomonas malvacearum did not infect the hypocotyls, but it did produce lesions on the cotyledons of susceptible varieties of cotton at 21° to 36°C. The lesions were somewhat more extensive in the 24° to 33° range than at 21° and 36°, although they were slightly more numerous at 36° than at the lower temperatures. Although this bacterium may not be a direct cause of damping-off, the lesions which it produces may retard the development of the seedlings; and thus may predispose the seedlings to infection by pathogens which may cause seedling losses.

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